Hemocompatibility testing in the 21st century:

Options and pitfalls

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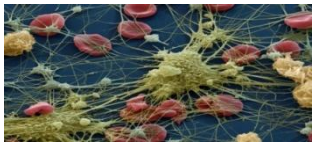
EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Eberhard-Karls-Universität
UKT
Universitätsklinikum Tübingen

Our research focus

Blood contacting biomaterials / Biologization of medical devices

GLP lab for hemocompatibility tests

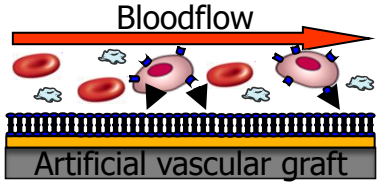


1. Accredited GLP test lab for „Testing of blood contacting medical devices accord. to ISO 10993-4“ with fresh human whole blood
2. Investigations in big animal models (pig, sheep)

In vitro pyrogen tests

Die innovative Pharmeuropa akzeptierte in-vitro Pyrogentestmethode.
Ein humanspezifisches Verfahren zur Prüfung auf Pyrogenfreiheit von Injektabilia und Medizinprodukten

In vivo Endothelialization

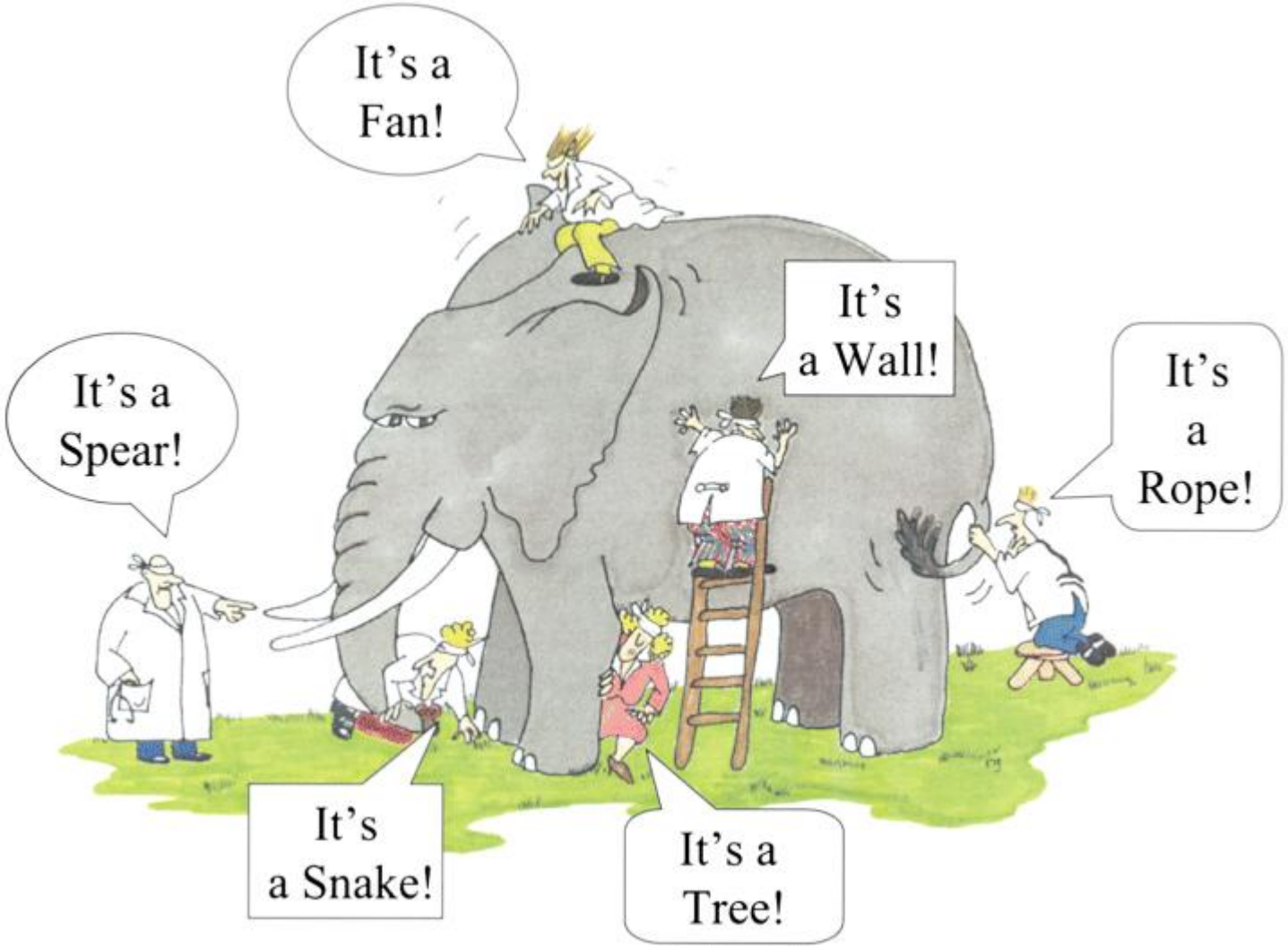


Fishing for Stem cells:
Mimicry of homing factors for in vivo cell seeding.
Hemocompatible polymer matrix with immobilized capture molecules for EPCs.

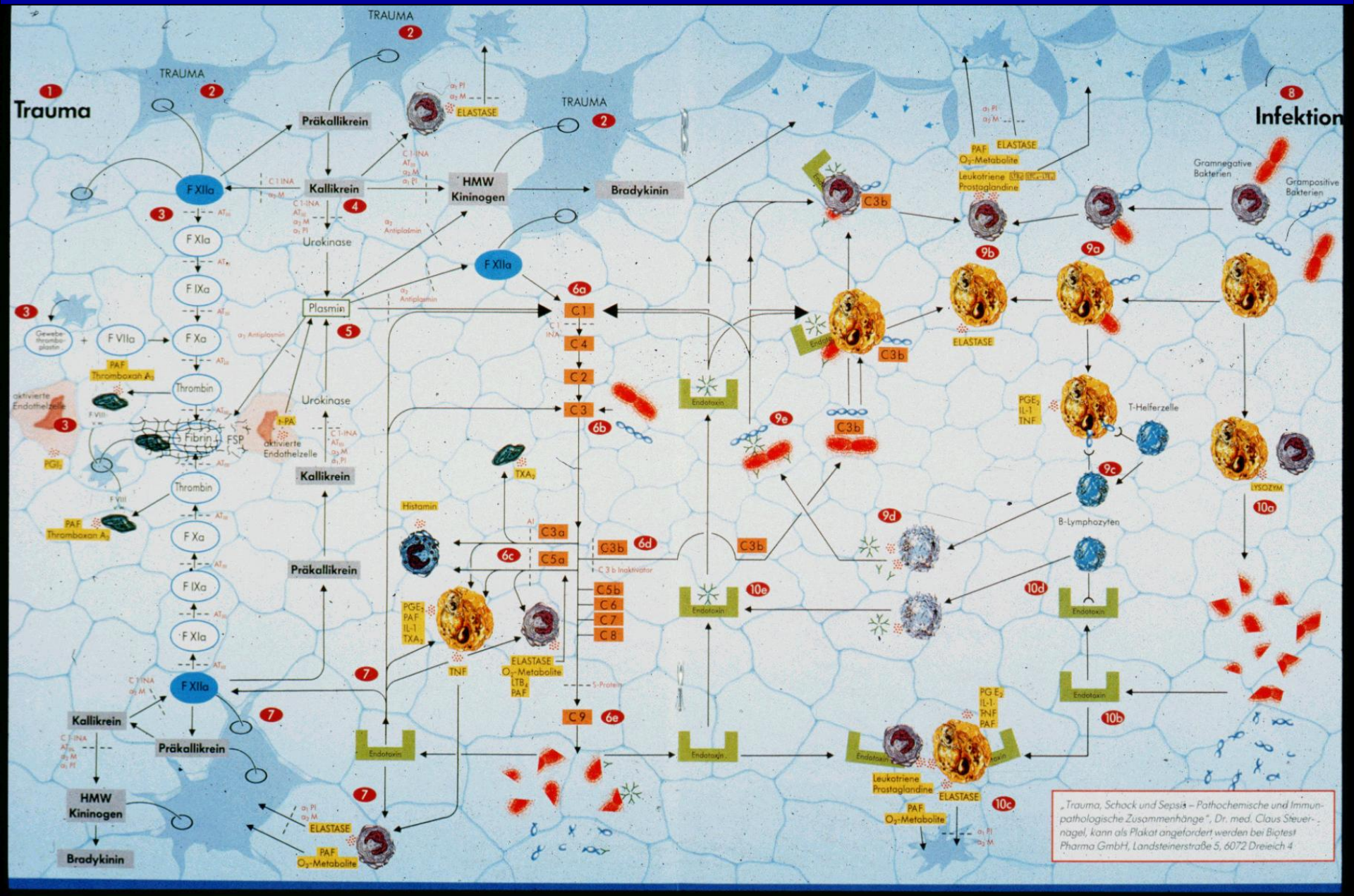
Gene Silencing Medical Devices

Next stent generation:
siRNA based coatings for local gene silencing
to reduce neointimal hyperplasia

What is Hemocompatibility?



Hemocompatibility or subway plan of London?



Buddy‘s view of the hemocompatibility problem

Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 28 (2007) 5144–5147
Biomaterials
www.elsevier.com/locate/biomaterials

Leading Opinion

The catastrophe revisited: Blood compatibility in the 21st Century☆, ☆☆

Buddy D. Ratner

University of Washington Engineered Biomaterials (UWEB), University of Washington, Seattle, WA 98195, USA

Received 15 June 2007; accepted 19 July 2007
Available online 8 August 2007

Abstract

The biomaterials community has been unable to accurately assign the term “blood compatible” to a biomaterial in spite of 50 years of intensive research on the subject. There is no clear consensus as to which materials are “blood compatible.” There are no standardized methods to assess blood compatibility. Since we use millions of devices in contact with blood each year, it is imperative we give serious thought to this intellectual catastrophe. In this perspective, I consider five hypotheses as to why progress has been slow in evolving a clear understanding of blood compatibility: Hypothesis 1—It is impossible to make a blood compatible material. Hypothesis 2—We do not understand the biology behind blood compatibility. Hypothesis 3—We do not understand how to test for or evaluate blood compatibility. Hypothesis 4—Certain materials of natural origin seem to show better blood compatibility but we do not know how to exploit this concept. Hypothesis 5—We now have better blood compatible materials but the regulatory and economic climate prevent adoption in clinical practice.

Buddy‘s view of the hemocompatibility problem

Hypothesis 1: It is impossible to make a blood compatible material.

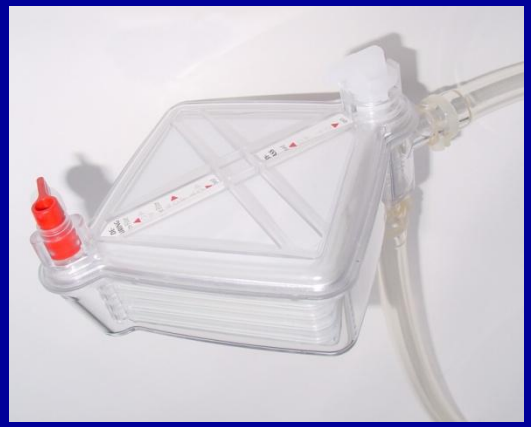
Hypothesis 2: We do not understand the biology behind blood compatibility.

Hypothesis 3: We do not understand how to test for or evaluate blood compatibility.

Hypothesis 4: Certain materials of natural origin seem to show better blood compatibility but we do not know how to exploit this concept.

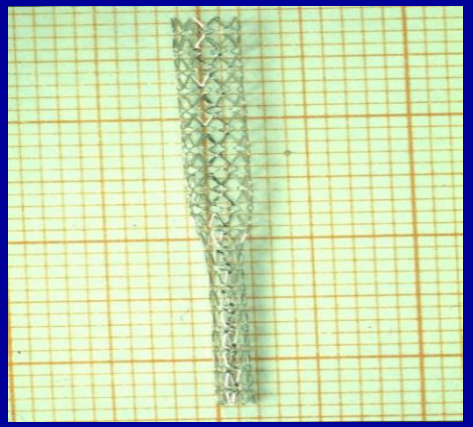
Hypothesis 5: We now have better blood compatible materials but the regulatory and economic climate prevent adoption in clinical practice.

Cardiovascular medical devices in blood contact
from minutes to whole life

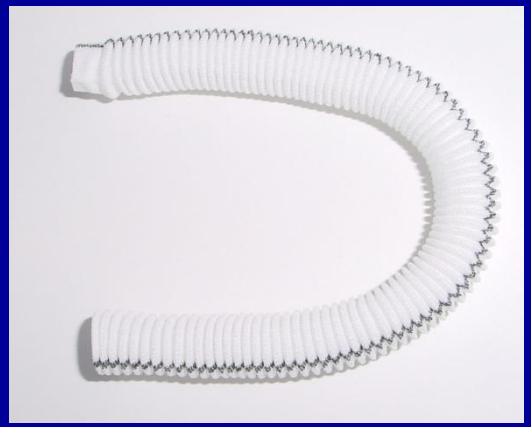


Art. Filter

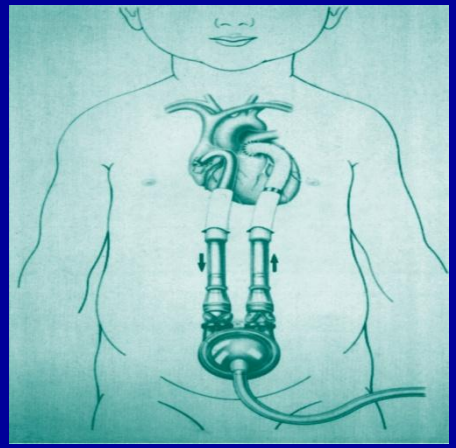
Oxygenator



Stents



Vascular Grafts



VAD

Heart valve

What do we expect from a blood compatible surface?

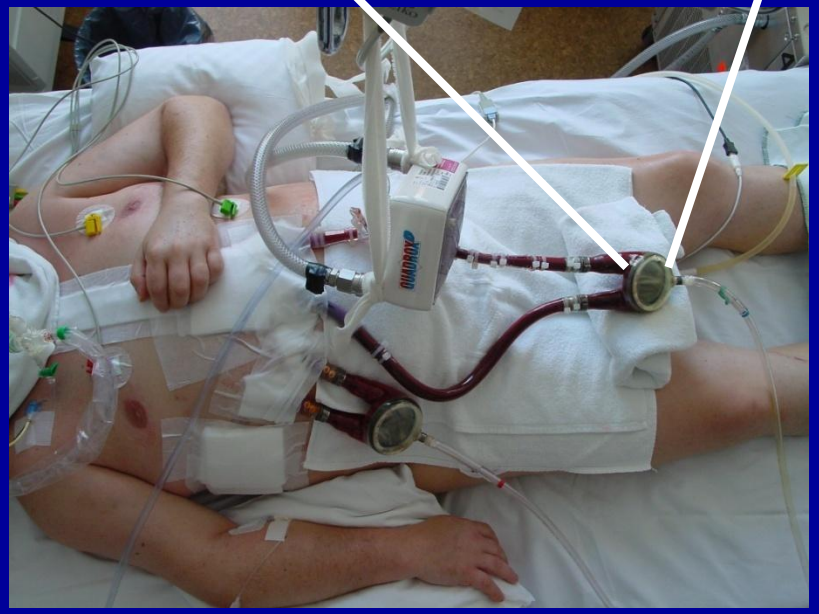
Technical functionality

+

- ✓ **No platelet adhesion**
- ✓ **Not thrombogenic**
- ✓ **Not pro inflammatory**
- ✓ **Pro healing**

Problem

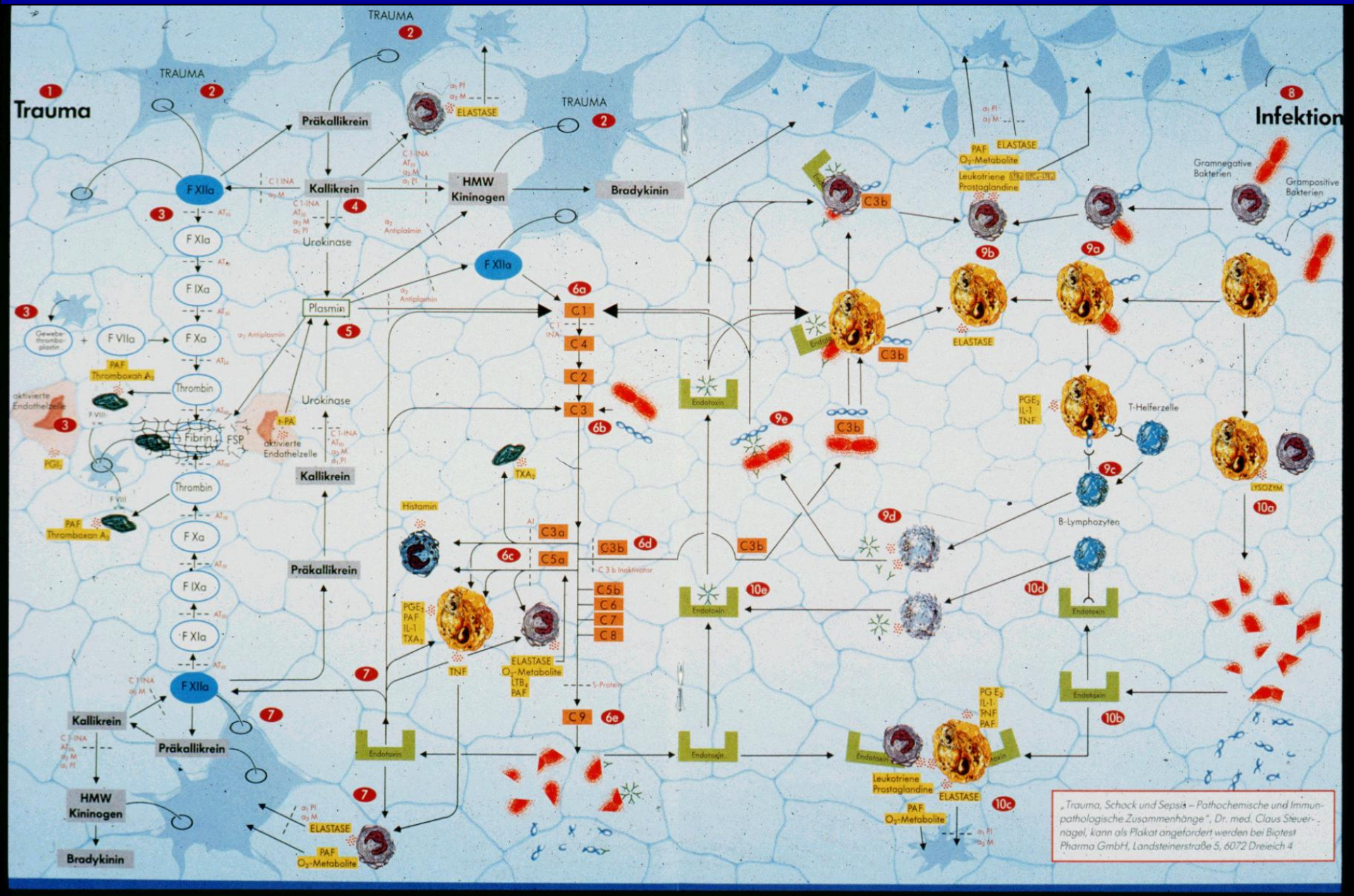
still poor
hemocompatibility
of the devices



"Blood is a very special juice"

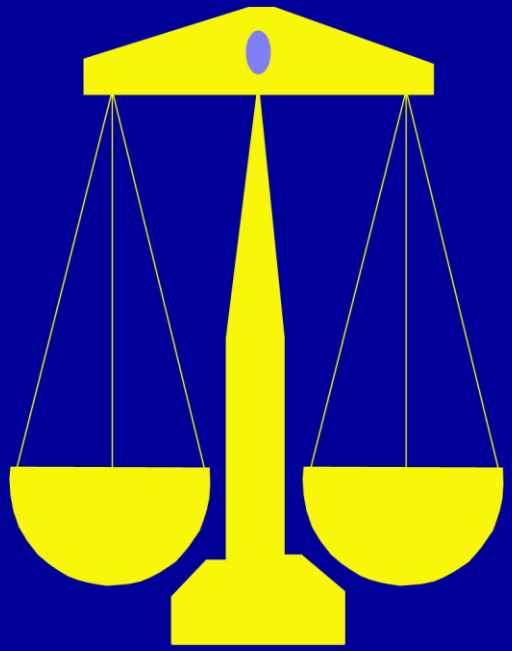
Mephisto to Dr. Faust in the Pact Scene (Faust I, J.W. v. Goethe)

Blood: the well organized chaos



Hemostatic Balance

Coagulation



Fibrinolysis

Thrombosis:

Myocardial Infarction
Stroke
Lung Embolism
Deep Venous Thrombosis

Bleeding:

Cerebral Hemorrhage
Inner Bleeding
Retinal Bleeding
Hematoma

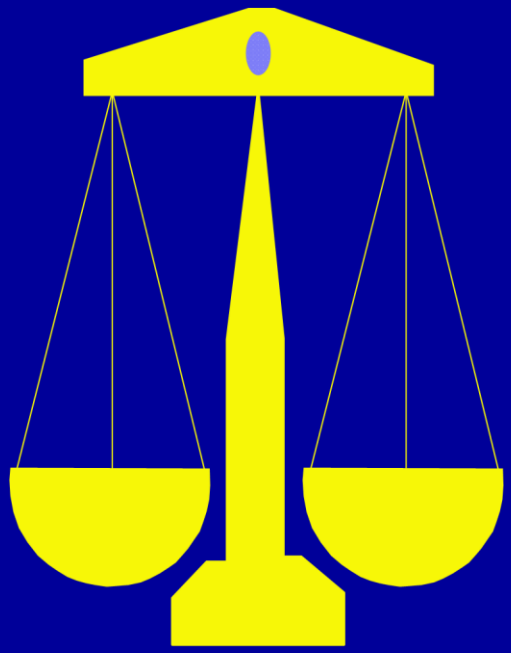
Hemostatic Balance

Activators:

Thromboplastin
(tissue factor)
Neg. Surfaces

Activators:

Kallikrein
t-PA
Streptokinase/r-t-PA



Coagulation

Fibrinolysis

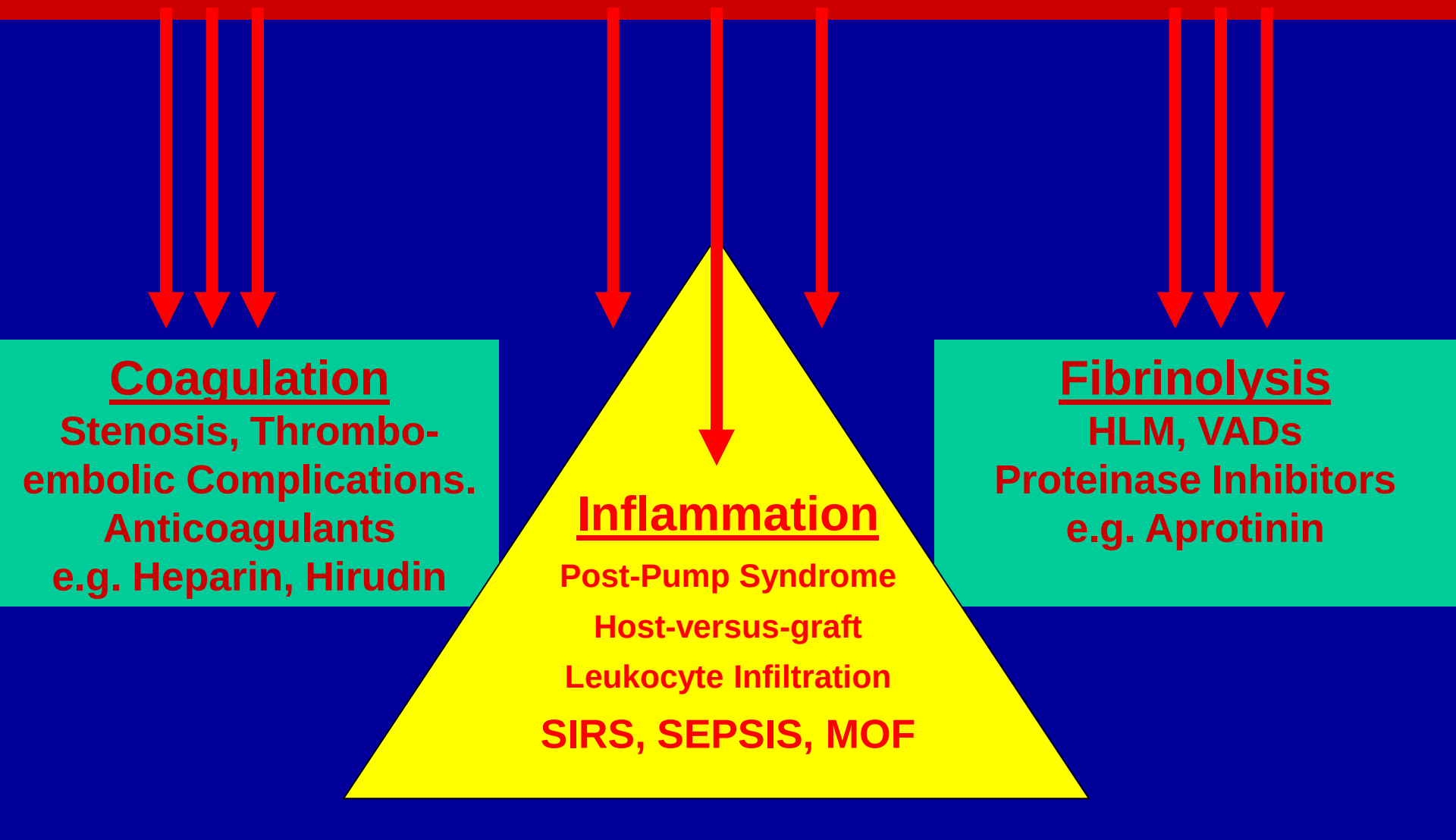
Inhibitors:

Heparin
Hirudin
Warfarin
ASS

Inhibitors:

Aprotinin
PAI
Antiplasmin

Problems Caused by Blood Contact with Artificial Surfaces



Models for in vitro hemocompatibility testing

Static models

Dynamic models

Going from small to big models

(Wim - Hans)

Hemocompatibility Testing
What kind of blood should be used?

<u>Human whole blood</u>

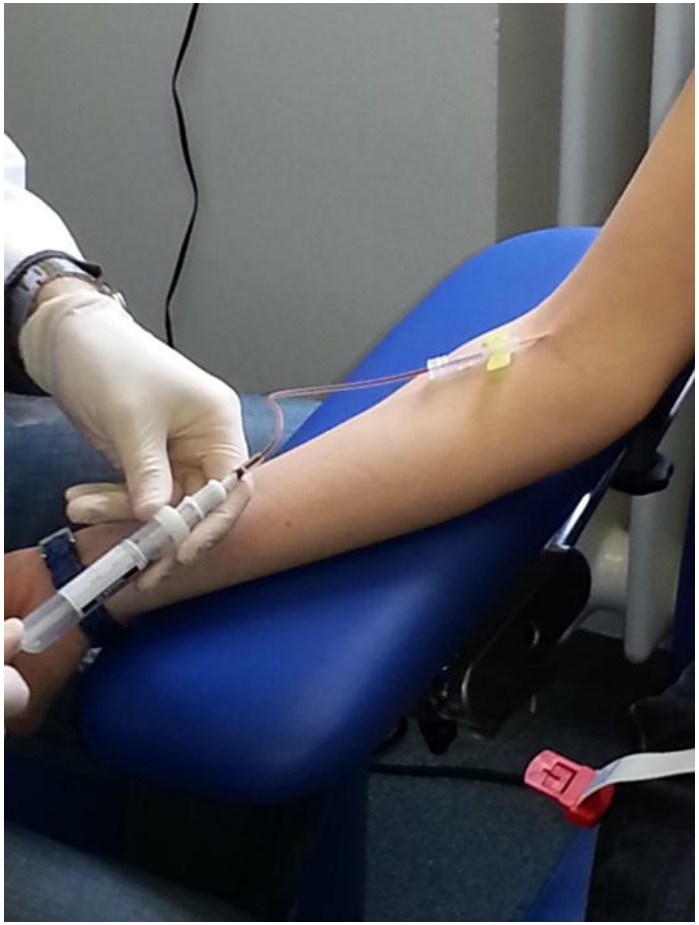
<u>directly from healthy blood donors</u>

1. **Fresh!**
2. **Fresh!**
3. **Fresh!**

<u>Exclusion Criteria:</u>

Smoking
Drug taking (Aspirin, Antiphlogistics, etc.)
Pregnancy, oral contraceptives

How to draw the blood?



Sterile!

No stasis, or only very short and soft stasis

Soft and slow filling

Do not produce a vacuum

Directly in containers prefilled with diluted anticoagulant

Shake them softly during donation

Need more blood for big models?

Use the transfusion service at the next corner

If not close enough:

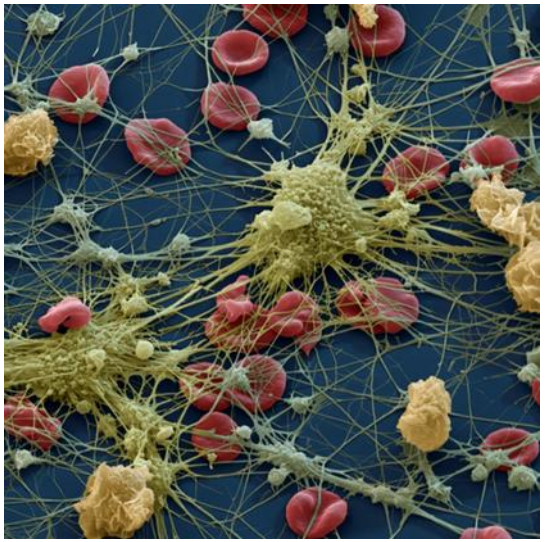
Forget it!

What kind of blood bags can be used?

Do not use blood bags from the transfusion service!

Use empty bags prefilled with your own anticoagulant

What kind of anticoagulation should be used?

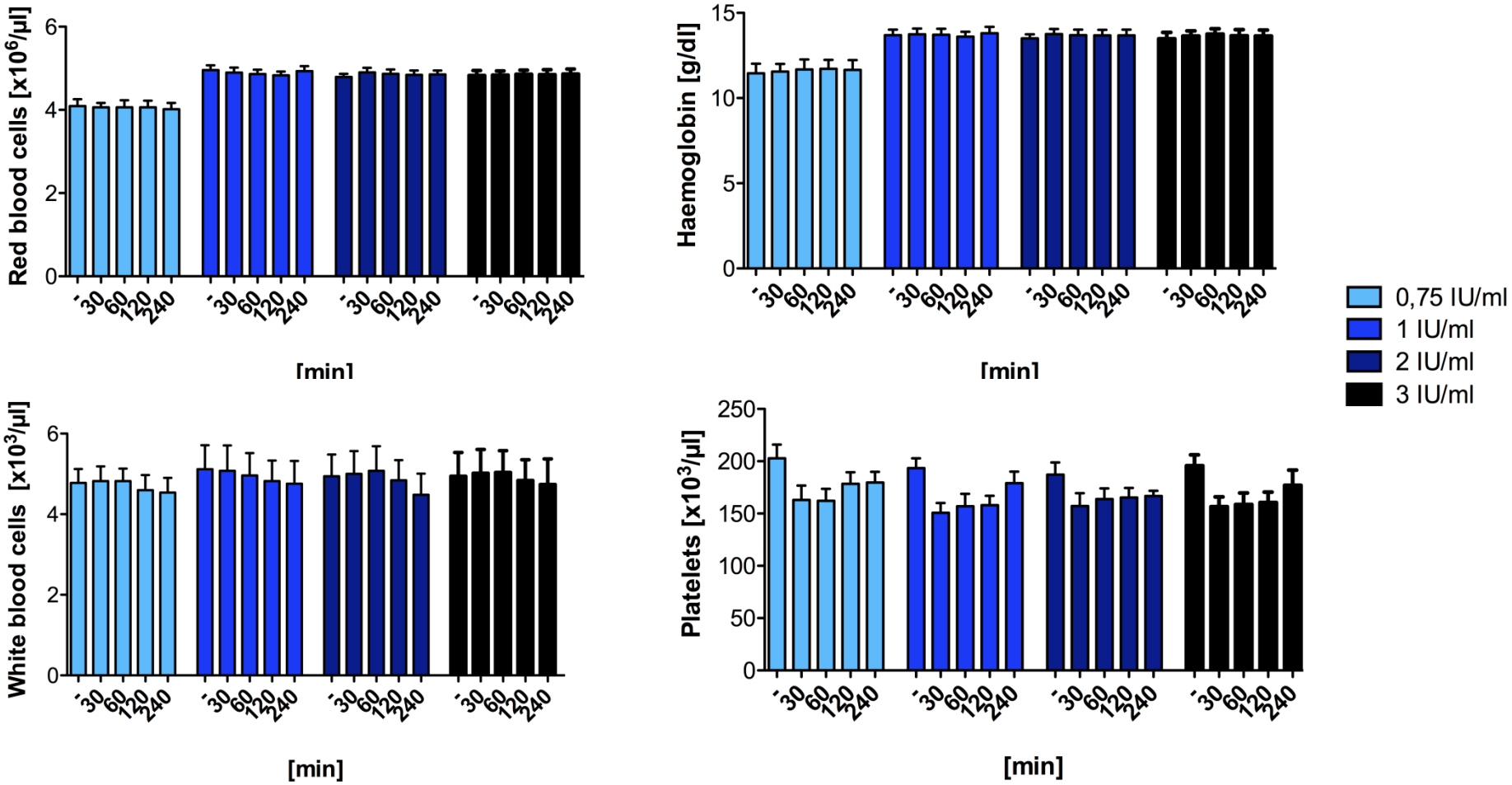


- **Use unfractionated heparin**
- **as less as possible or similiar to clinical application**

- **No citrated blood**
- **No hirudin**
- **No other anticoagulants**

Comparison of different heparin concentrations

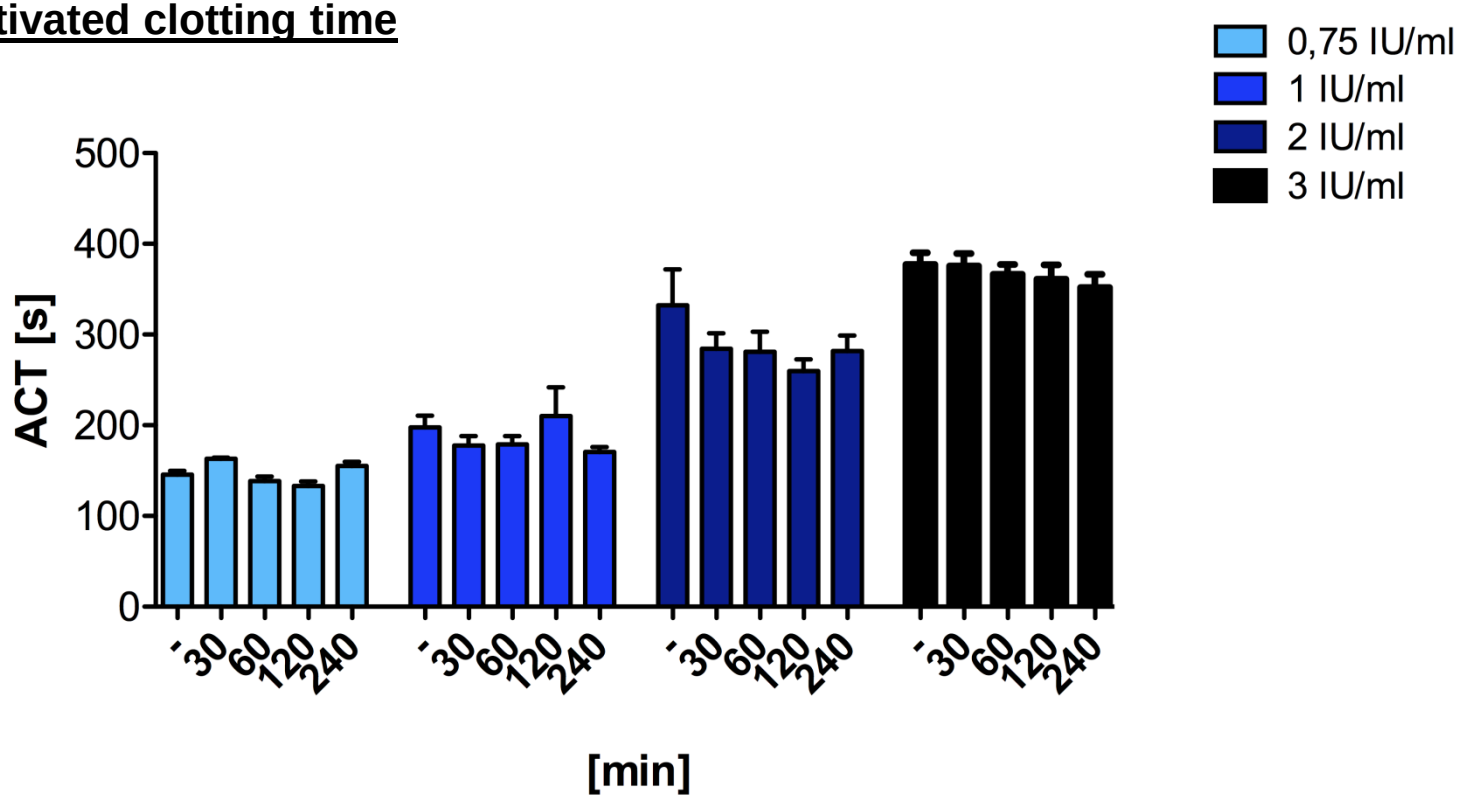
Blood cell analysis



Human whole blood (n=5) was circulated in a Chandler loop model for 30, 60, 120 or 240 minutes at 37°C

Comparison of different heparin concentrations

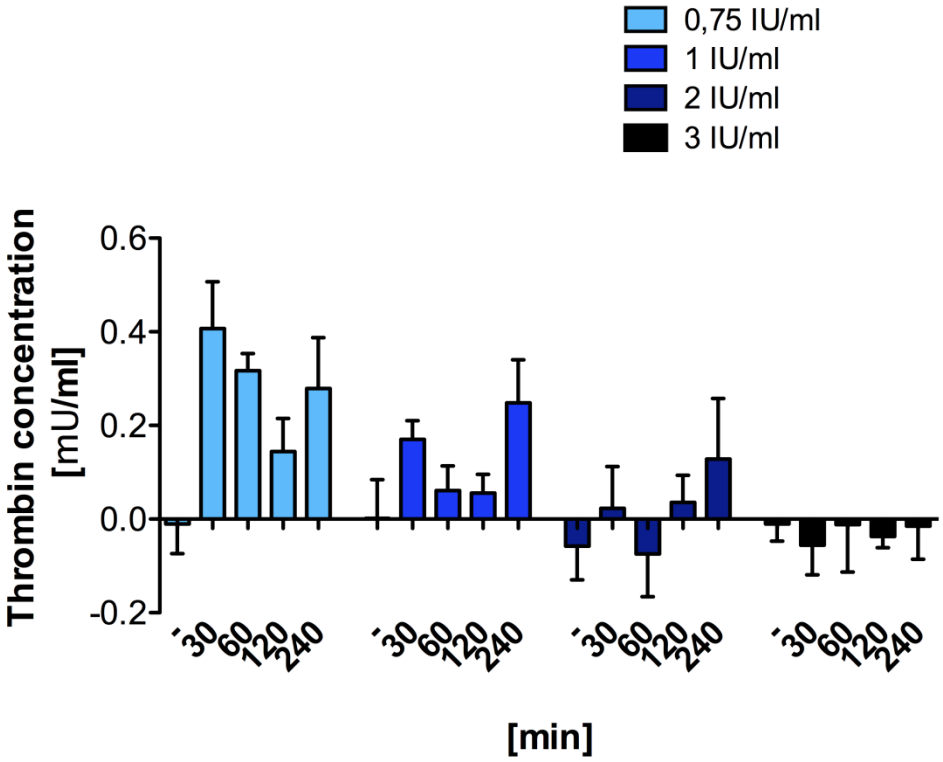
Activated clotting time

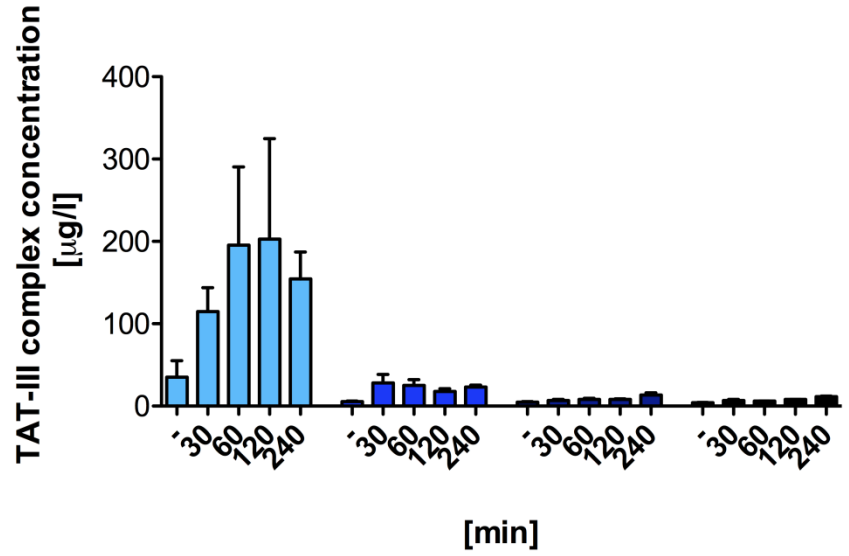


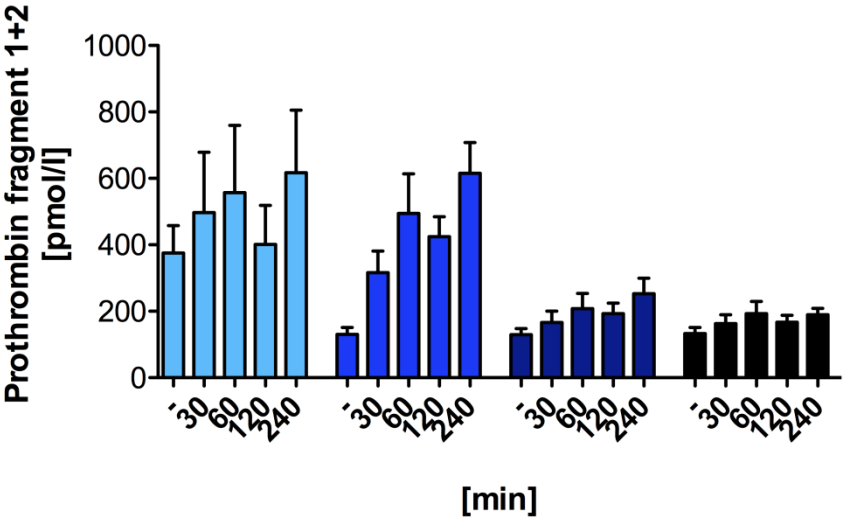
Human whole blood (n=5) was circulated in a Chandler loop model for 30, 60, 120 or 240 minutes at 37°C

Comparison of different heparin concentrations

Thrombin generation

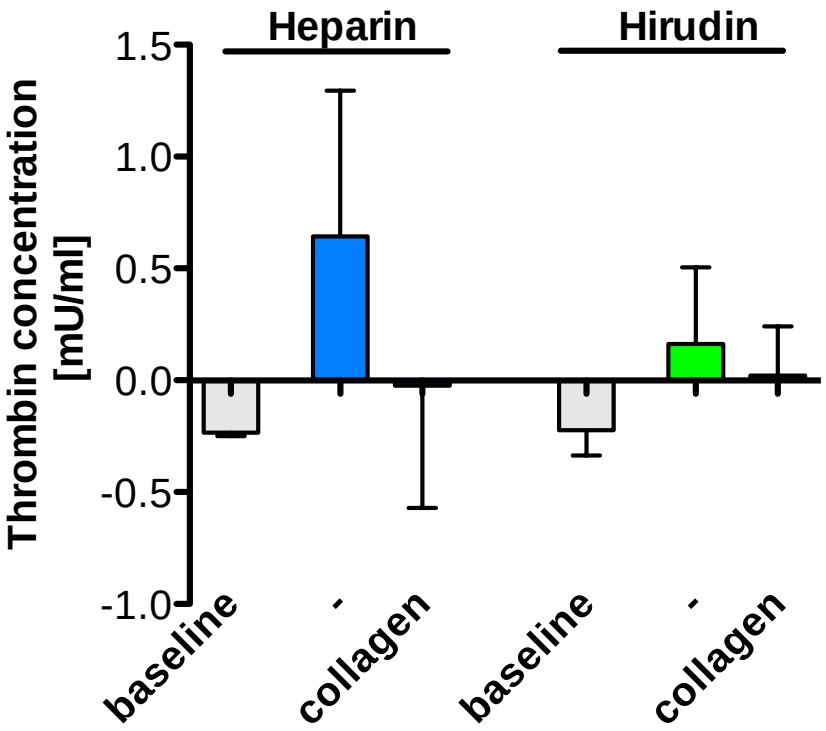


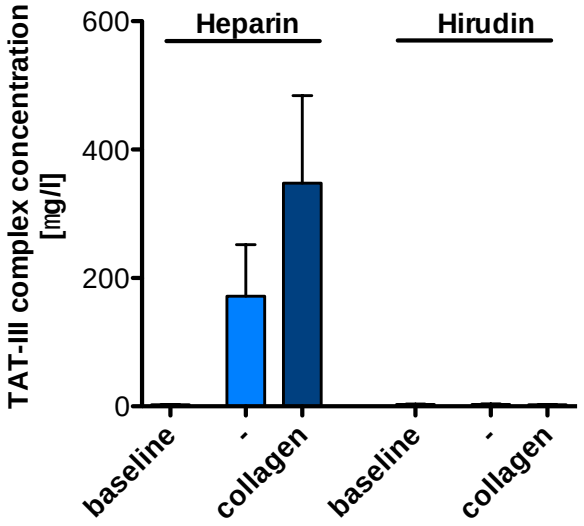


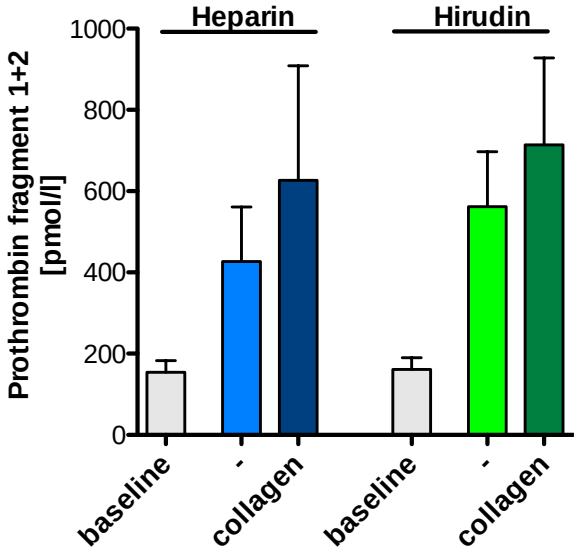


Human whole blood (n=5) was circulated in a Chandler loop model for 30, 60, 120 or 240 minutes at 37°C

Comparison heparin (1.0 IU/ml) vs. hirudin (50 µg/ml)







Human whole blood (n=3) was circulated in in a rotator model for 30 minutes at 37°C

What kind of predicates should be used?

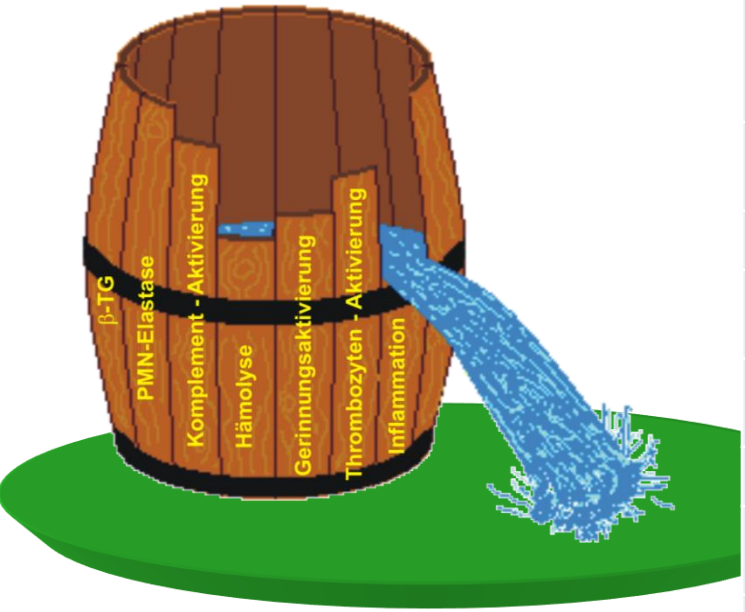
- Devices which are already on the market
- with comparable surface area

- Keep background activation low!

- If you are testing small surfaces (i.e. stents)
- Use heparin coated tubing

Activation Markers acc. to ISO 10993.4

Barrel stave theory



Test Category	Evaluation Procedure	Determination	Test Principle	Manufacturer
1. Thrombosis	SEM (scanning electron microscopy)	Platelet adhesion and aggregation, leukocyte adhesion, fibrinogen adsorption	Microscopy	Zeiss, EVOLS 10, Oberkochen, Germany
2. Coagulation	Marker for thrombin generation	Thrombin-Antithrombin-III complex (TAT)	ELISA	Siemens Healthcare Diagnostics Products, Marburg, Germany
3. Platelets	Number of platelets	Blood cell counting	Cell Counter Micros 60	ABX Hematology, Montpellier, France
	Marker for platelet activation	ß-Thromboglobulin	ELISA	Diagnostica Stago/Roche, Mannheim, Germany
4. Hematology	Number of white and red blood cells	Blood cell counting (leukocytes, erythrocytes, Hb, Hk)	Cell Counter Micros 60	ABX Hematology, Montpellier, France
	Hemolysis	Free plasma hemoglobin	Colorimetric assay	Cyan hemoglobin test, UKT, Germany
	Products of leukocyte activation	PMN-Elastase	ELISA	Demeditec Diagnostcs GmbH, Kiel, Germany
5. Complement system	Marker for activation of the C3 complement factor	C3a	ELISA	Quidel, San Diego, CA, USA
	Marker for activation of the terminal complement complex	SC5b-9	ELISA	Quidel, San Diego, CA, USA

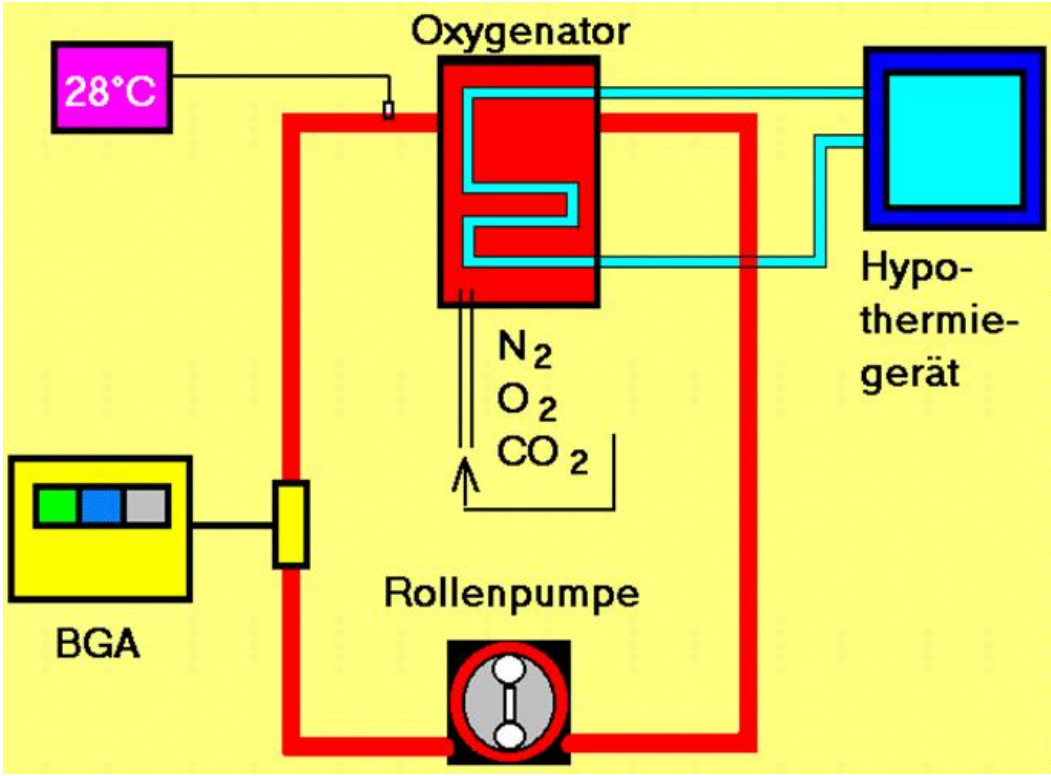
The most poor marker limits the overall performance

Do not use a score system

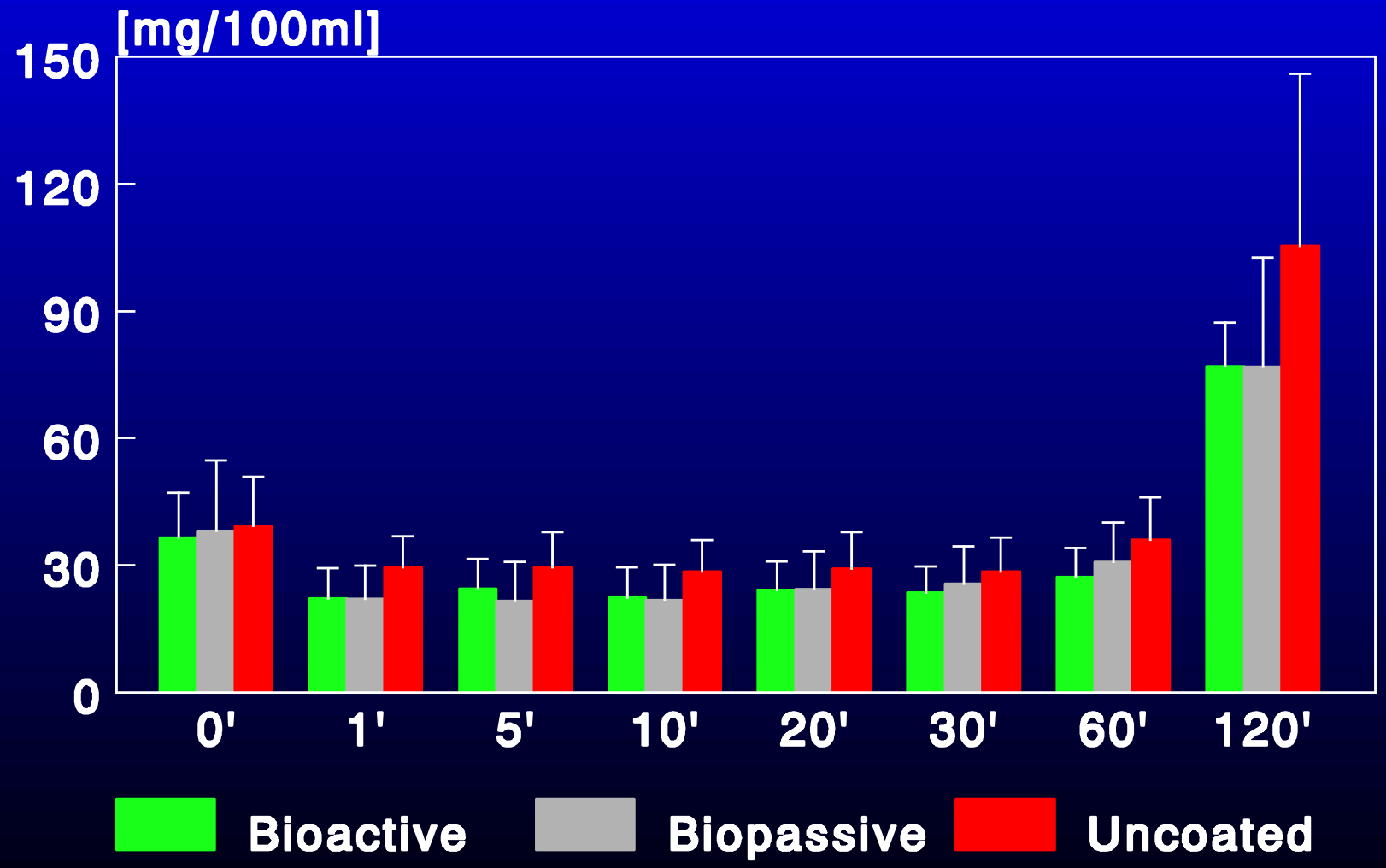
Example for in vitro oxygenator tests

Heart-Lung-Machine Model

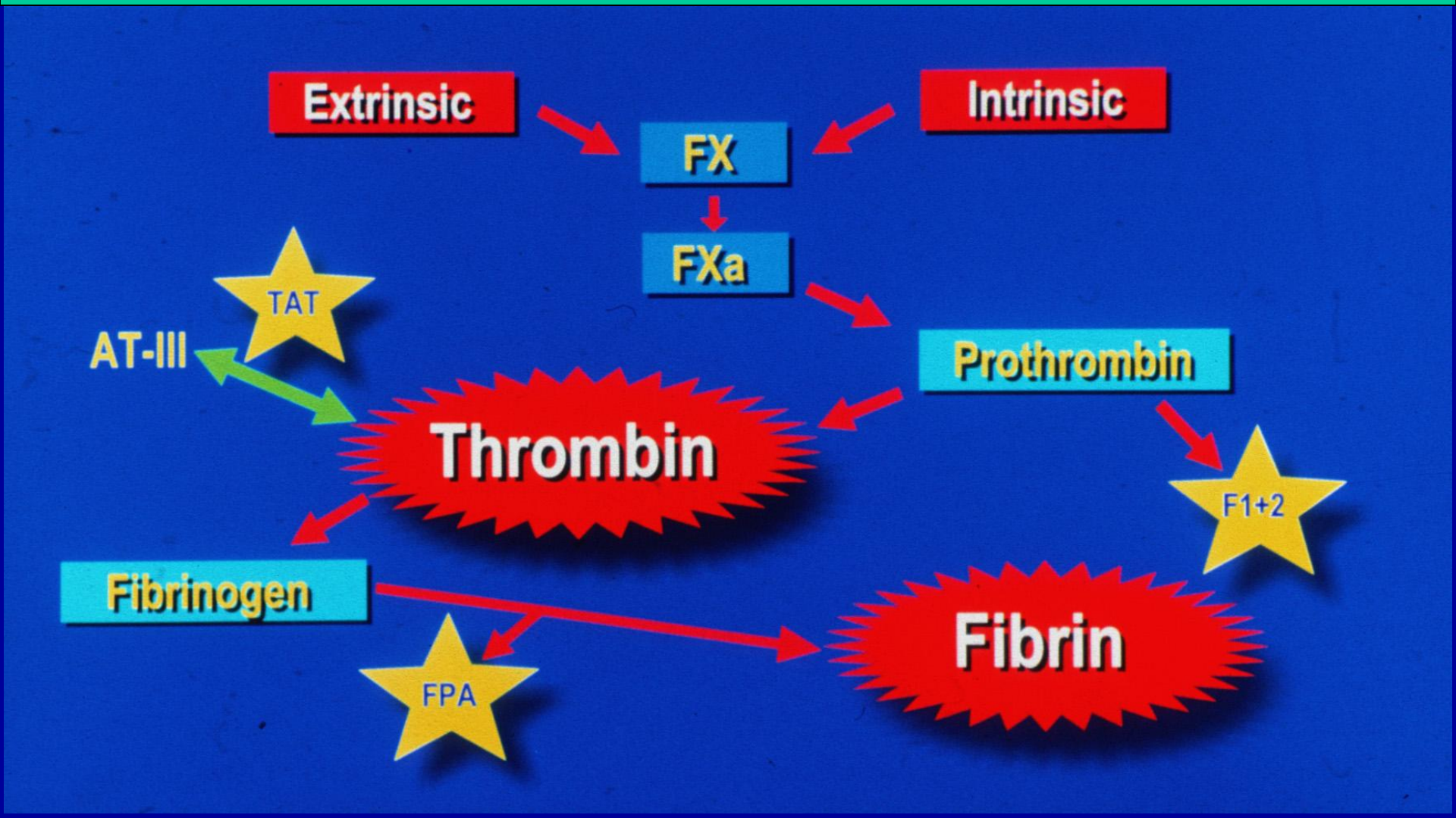
1. Uncoated oxygenator
2. Biopassive Coating
3. Bioactive Coating



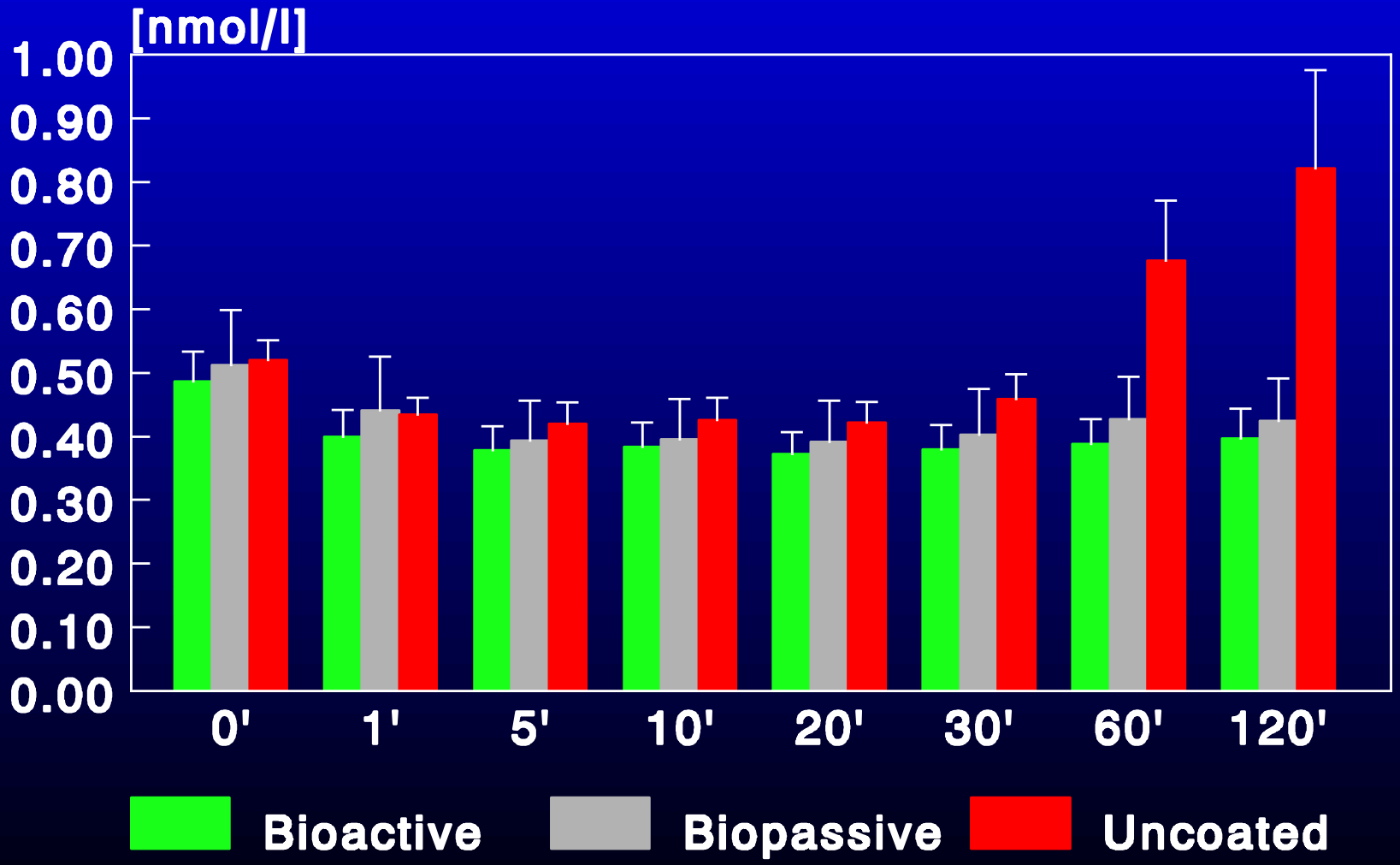
Hemolysis


[mg/100ml]
150
120
90
60
30
0
0'
1'
5'
10'
20'
30'
60'
120'
Bioactive
Biopassive
Uncoated

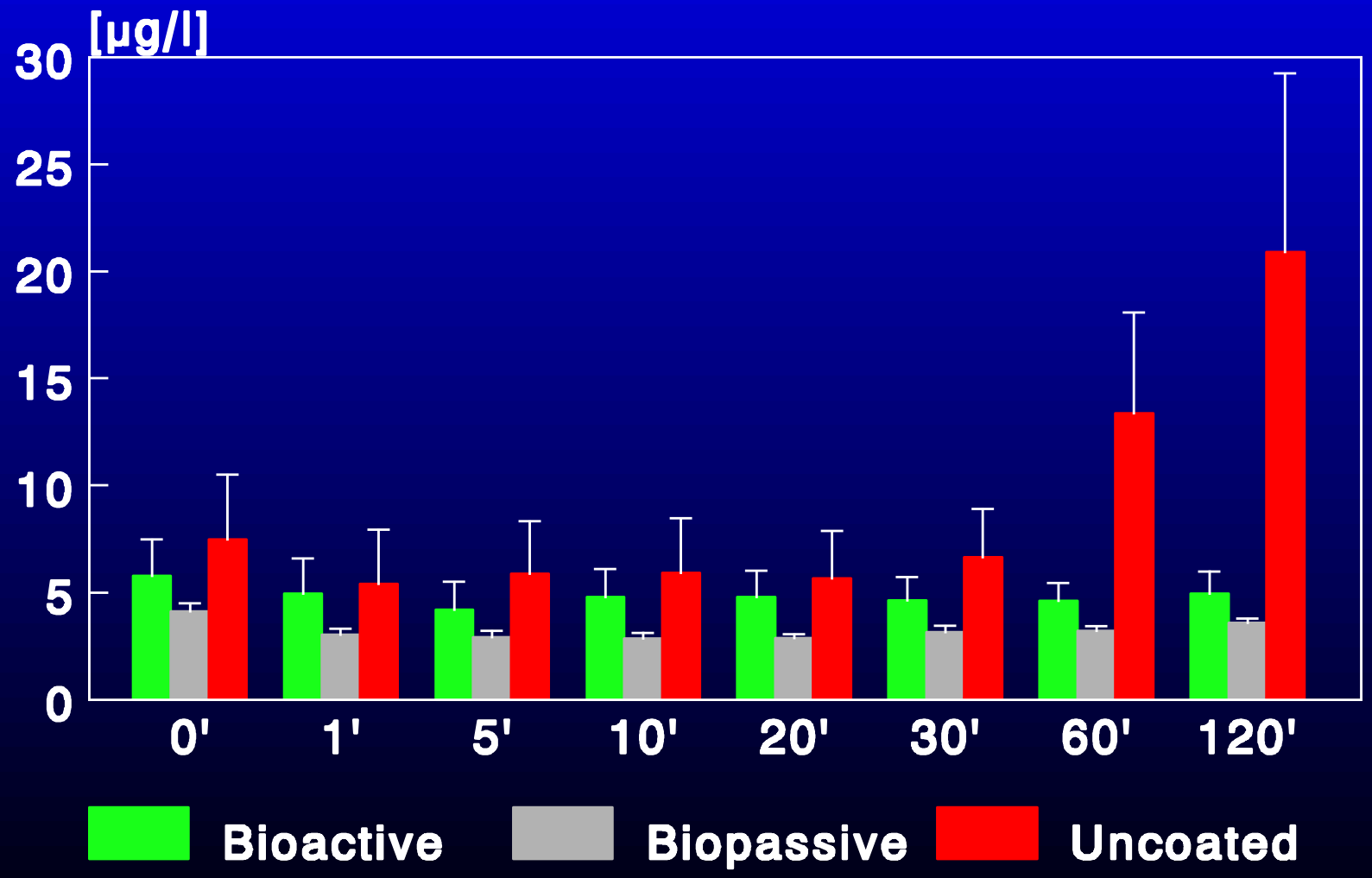
Final Coagulation Cascade



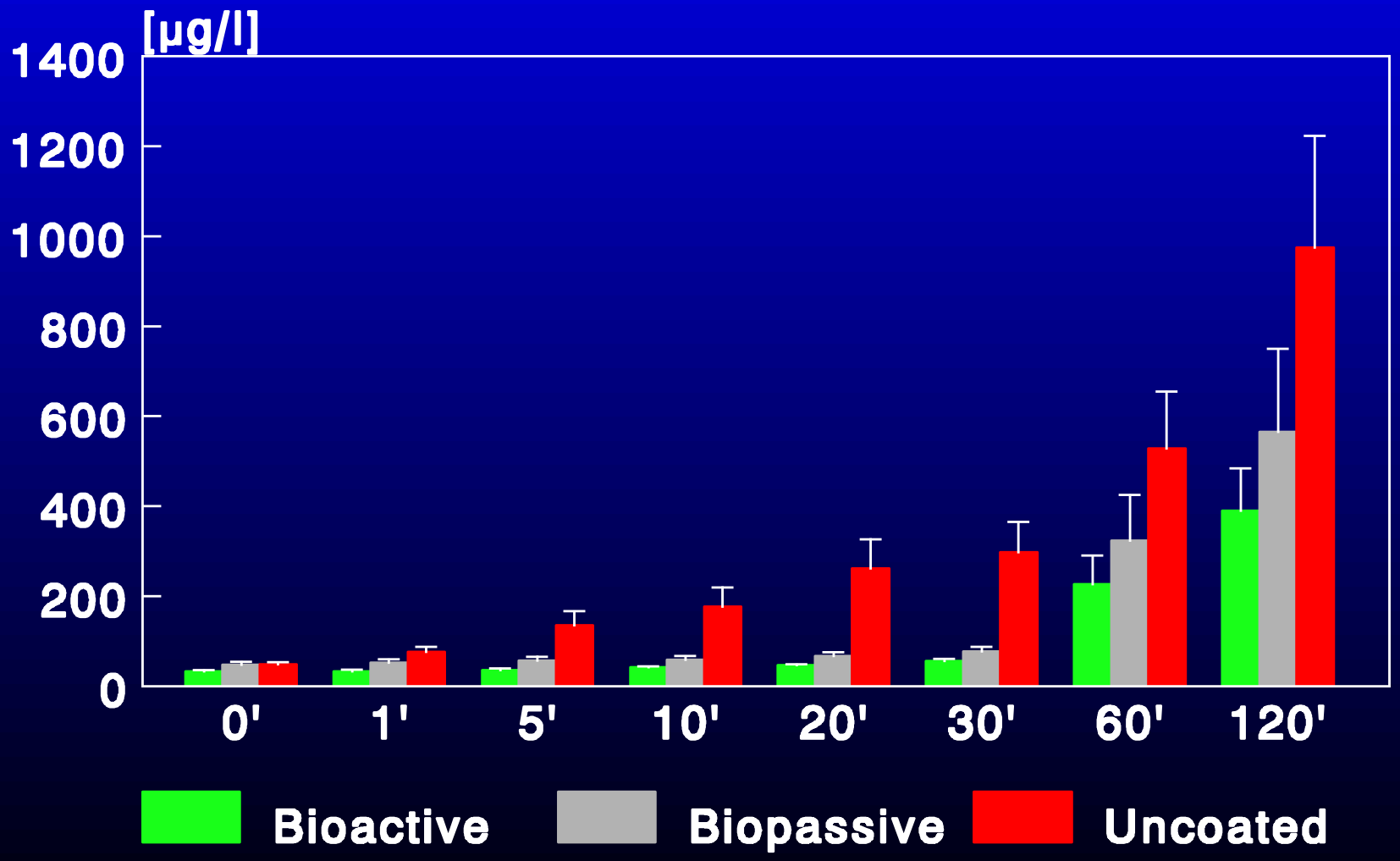
Prothrombinfragment 1+2



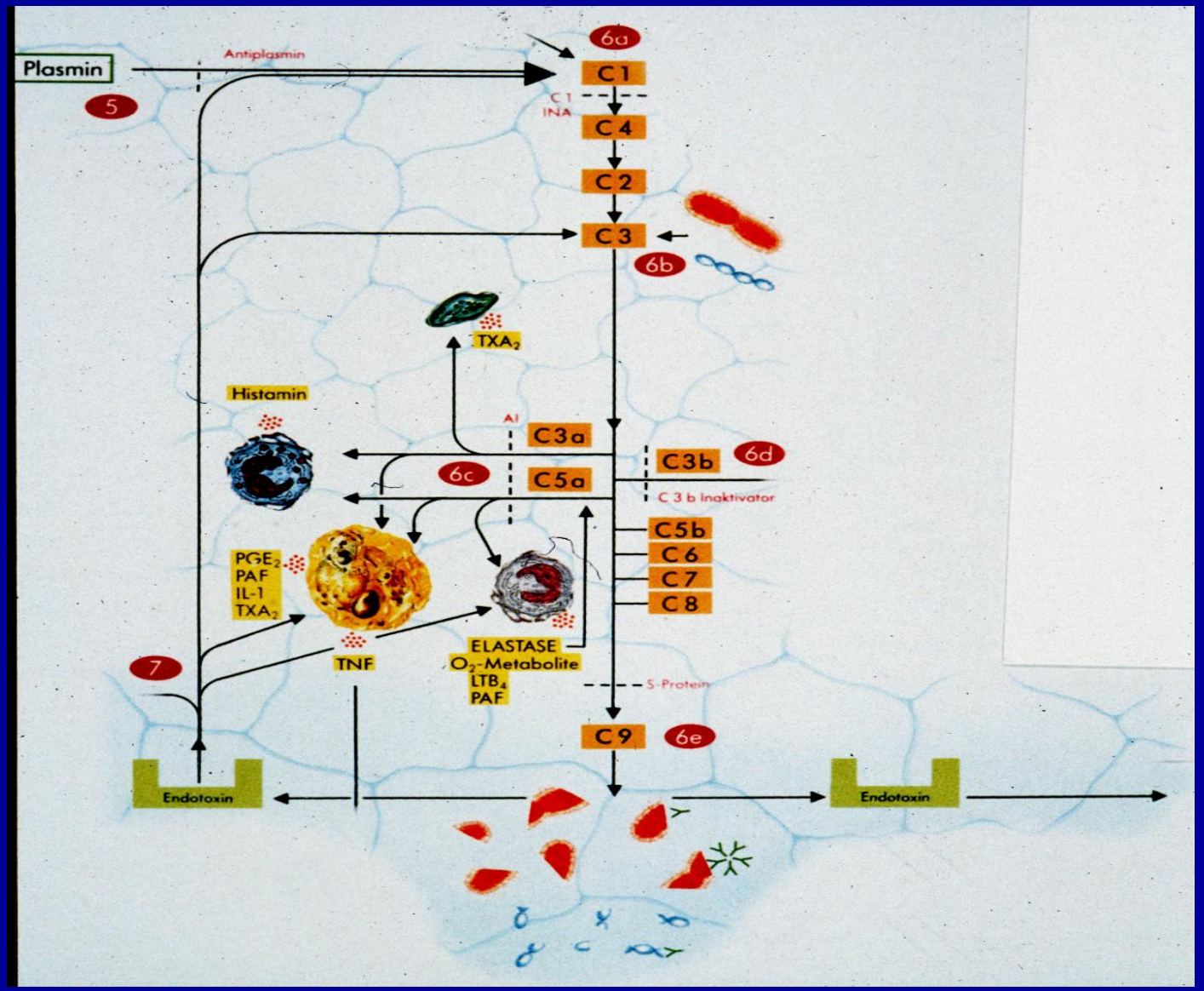
Thrombin-Antithrombin III-Complex



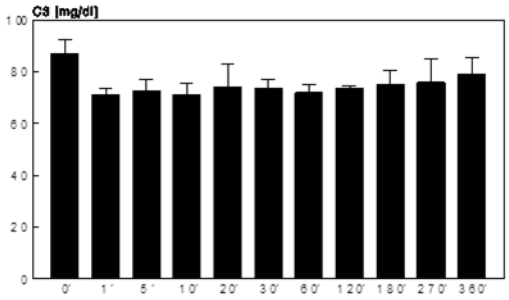
PMN-Elastase

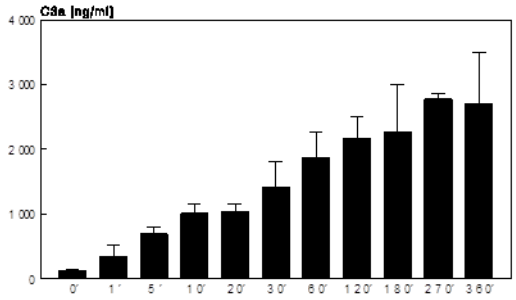


Complement System

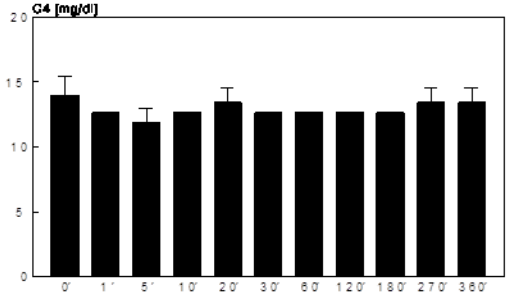


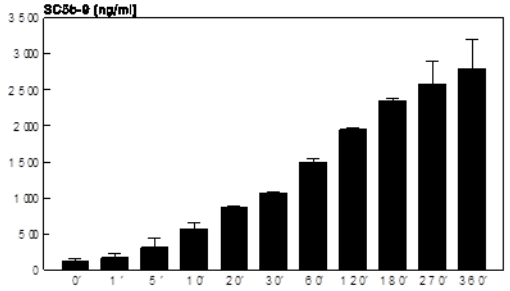
What complement factors should be looked for?





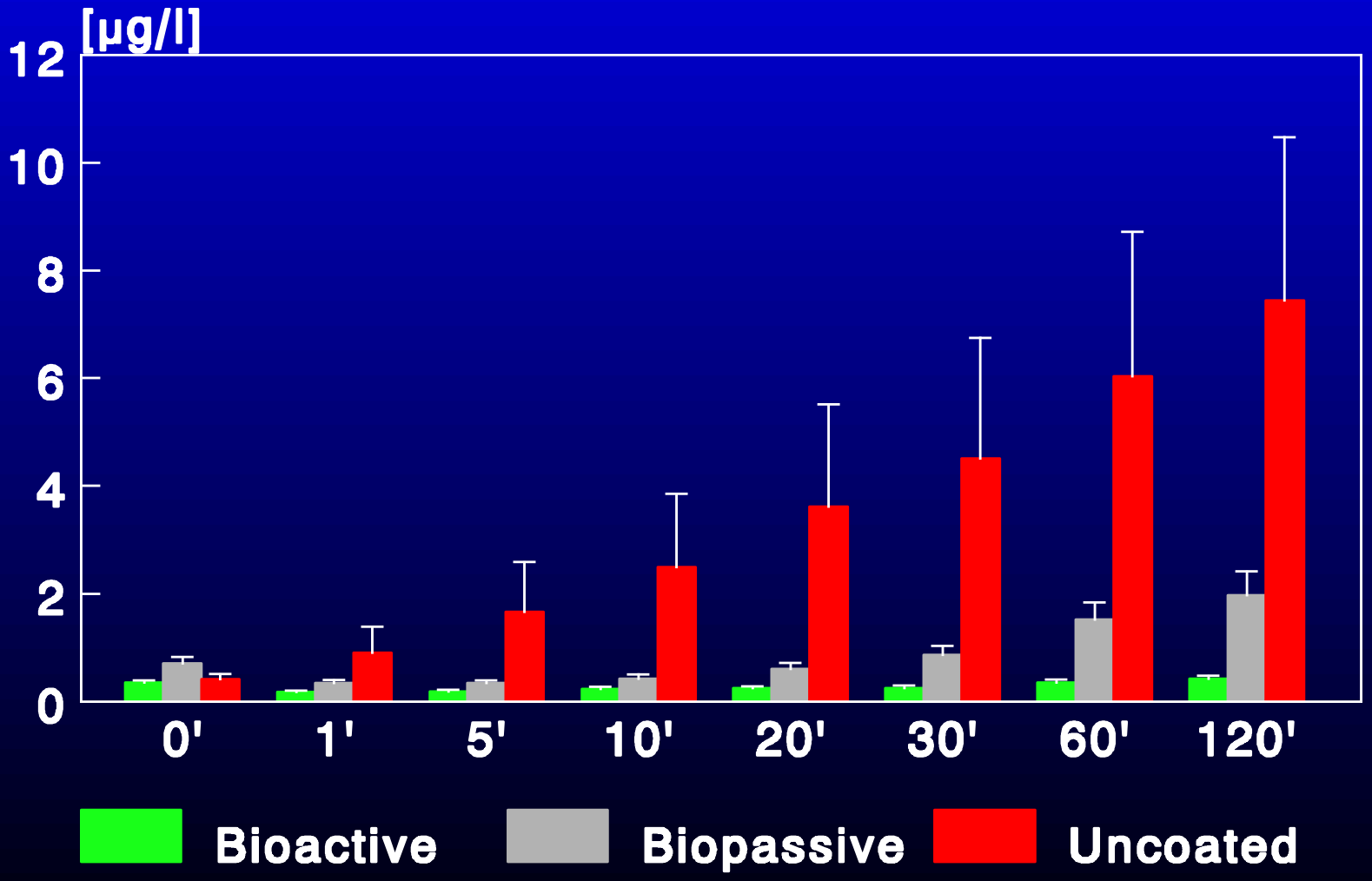
Comparison of C3 (immunologic) with C3a (ELISA)



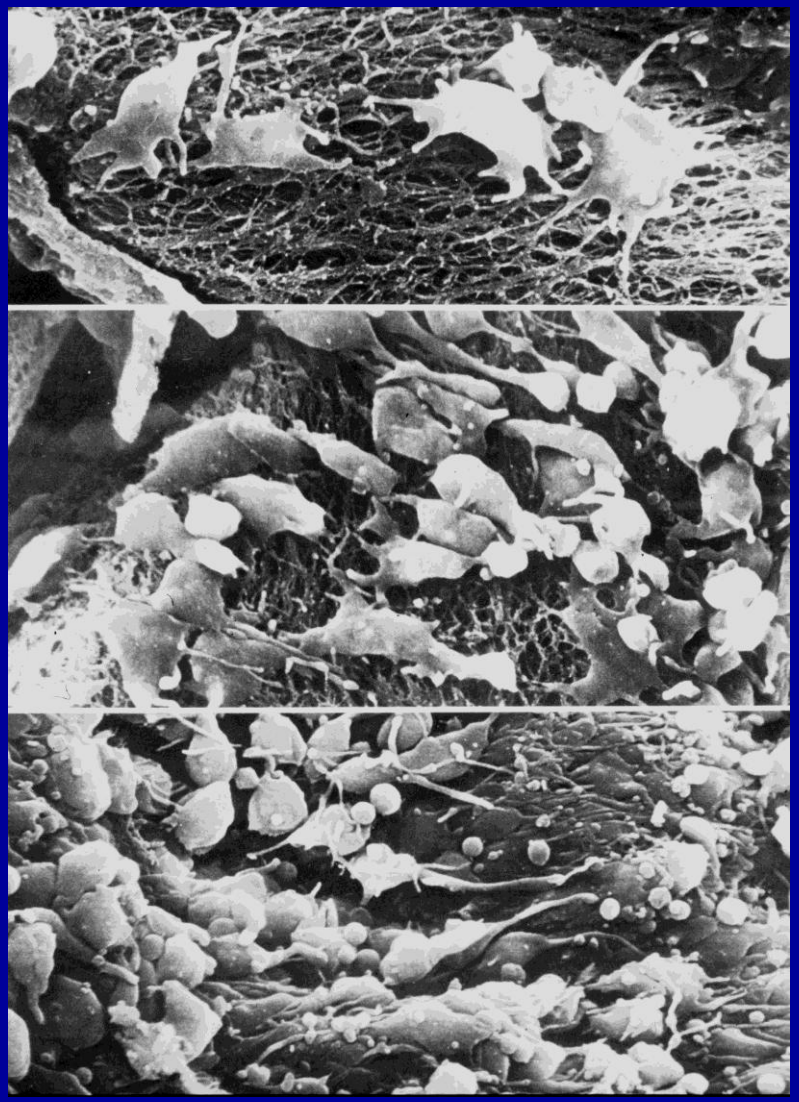


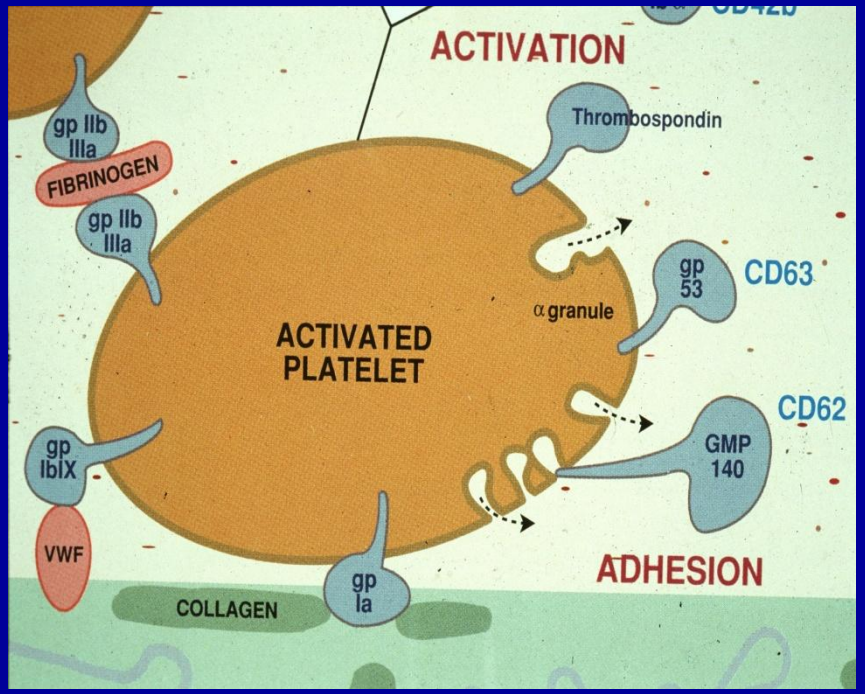
Comparison of C4 (immunologic) with SC5b-9 (ELISA)

C5a

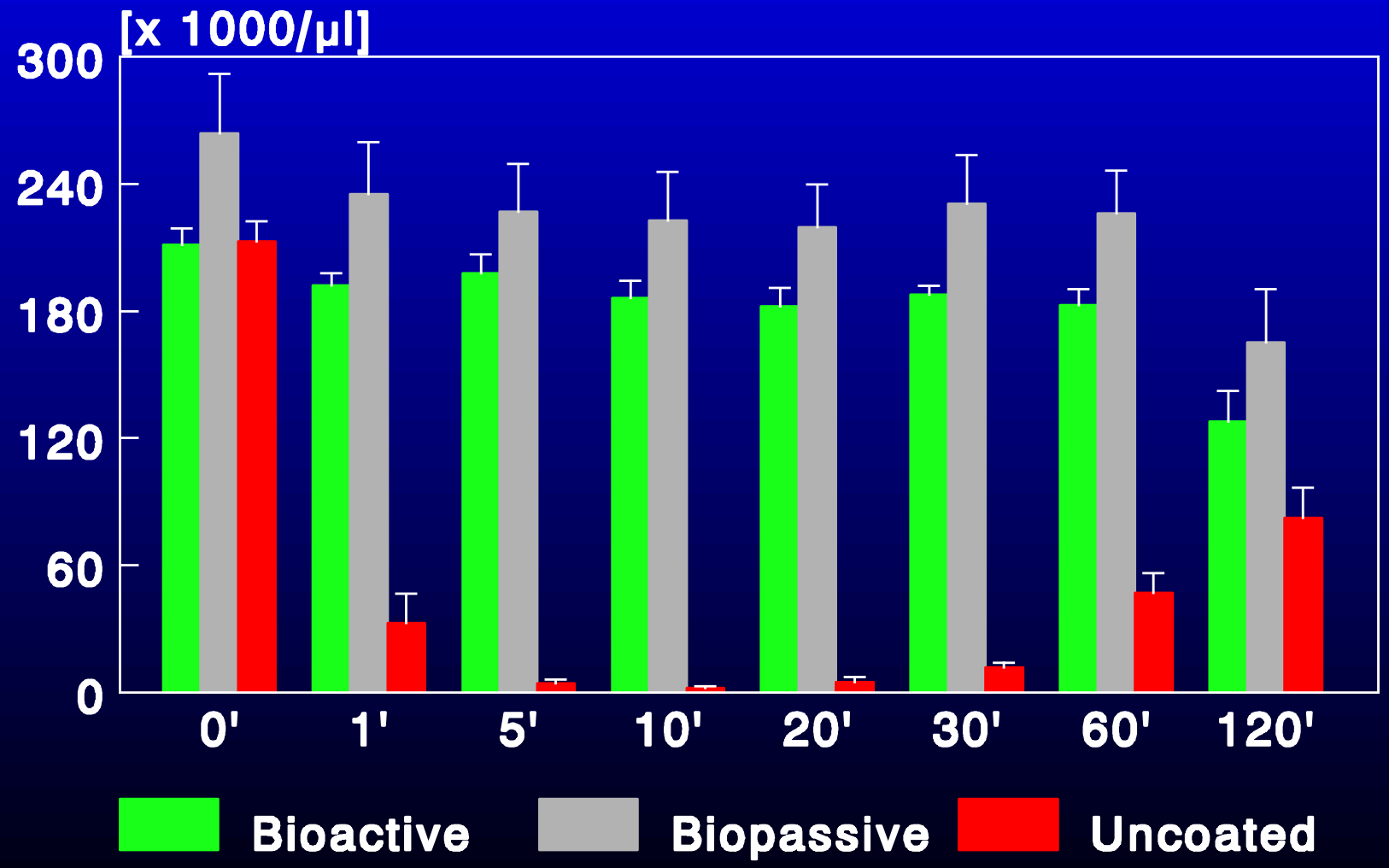

[µg/l]
12
10
8
6
4
2
0
0'
1'
5'
10'
20'
30'
60'
120'
Bioactive
Biopassive
Uncoated

Platelet Activation





Platelets


[x 1000/µl]
300
240
180
120
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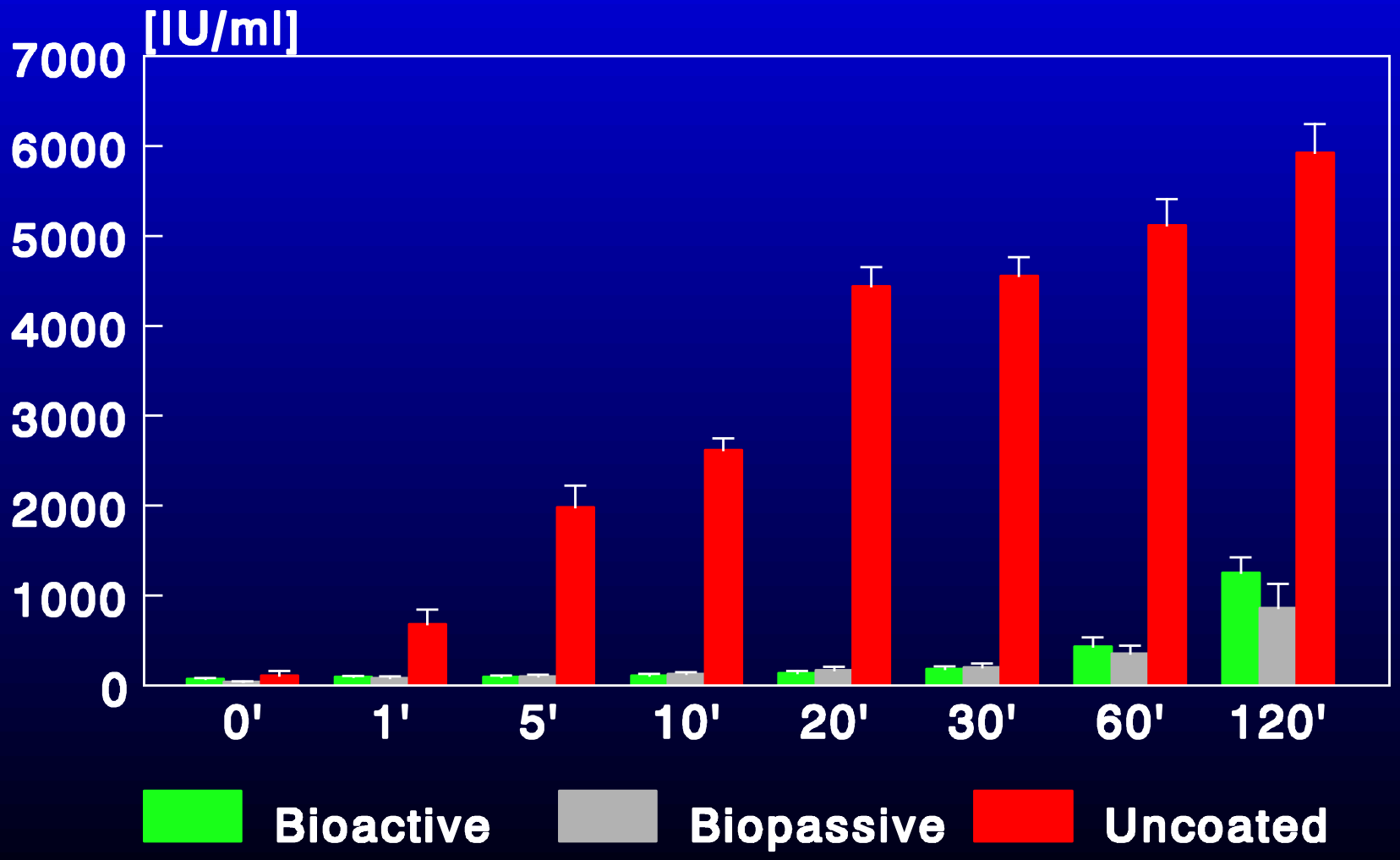
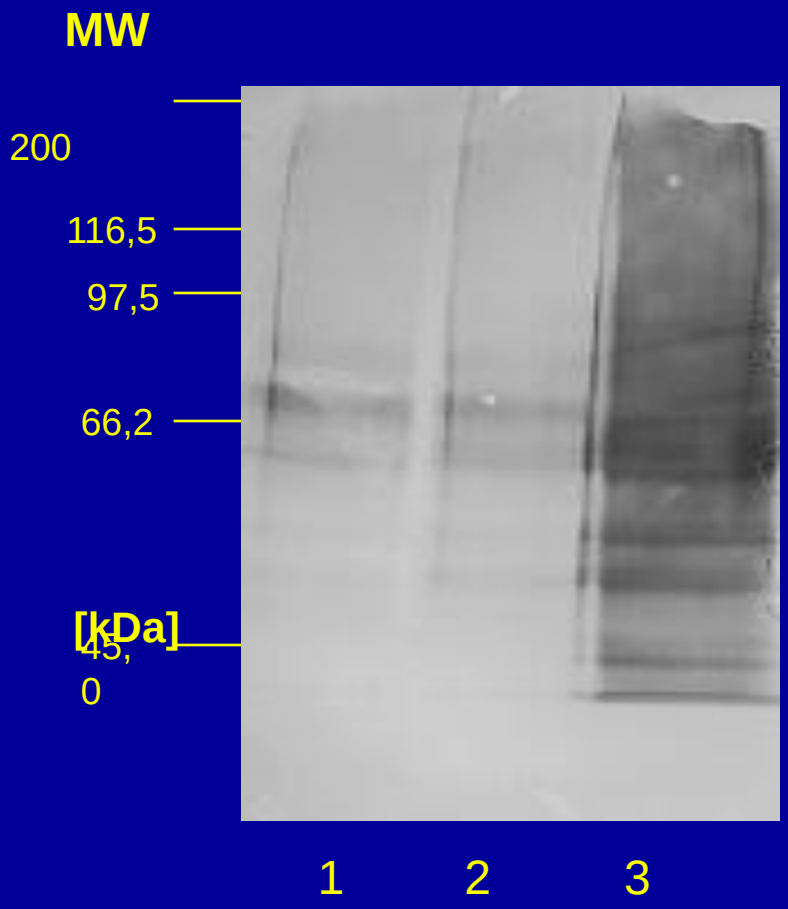
1'
5'
10'
20'
30'
60'
120'
Bioactive
Biopassive
Uncoated

ß-Thromboglobulin

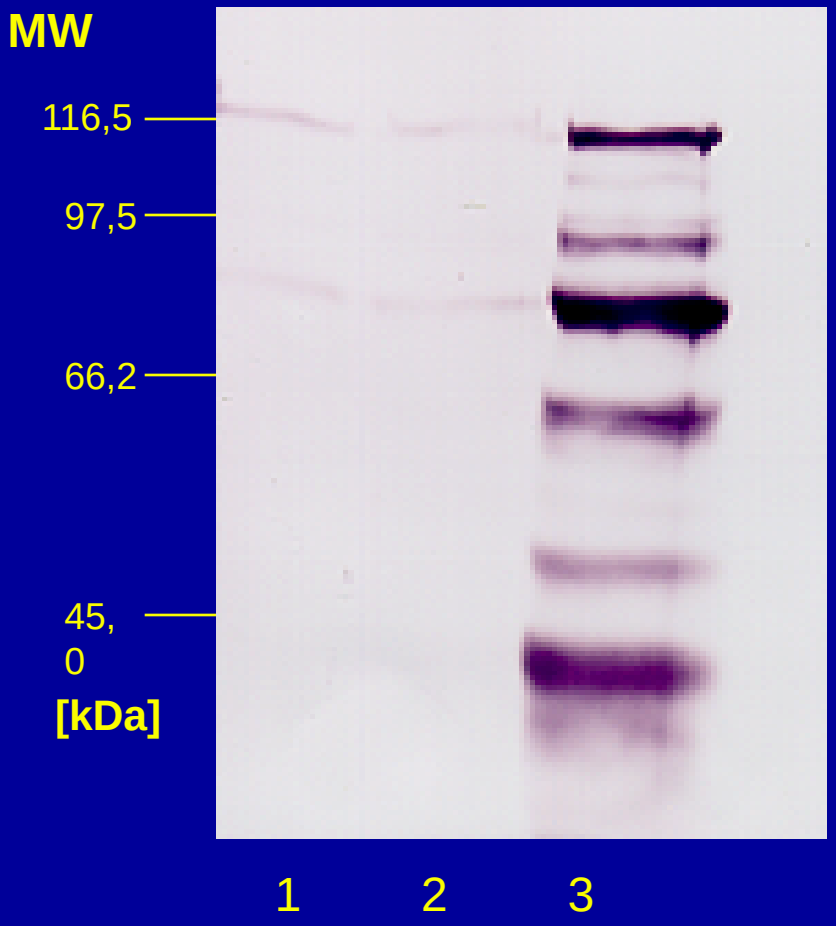


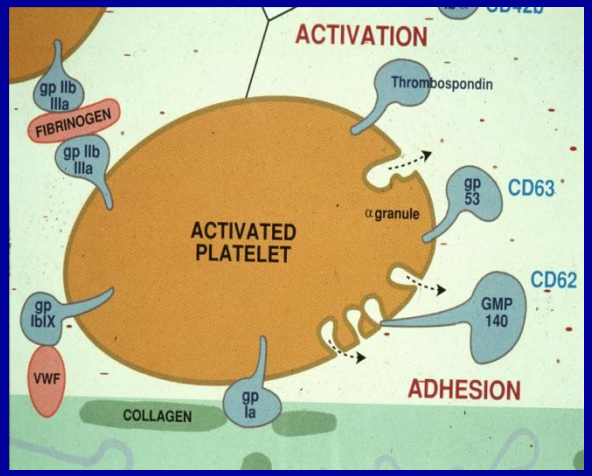
Protein Adsorption
Fibrinogen Western Blotting



1. **Biopassive**
2. **Bioactive**
3. **Control**

CD 41 Western Blotting





1. **Biopassive**
2. **Bioactive**
3. **Control**

Summary

With appropriate test models and usage of fresh human whole blood you can pefectly screen the hemocompatibility of medical devices in an early preclinical stage of device development

Tests for quality assurance of blood contacting medical devices

Short term animal experiments seem to be less sensitive compared to in vitro testing with fresh human blood

Thank you for your attention!